

U.S.S.N. 09/768,155

Filed: January 23, 2001

**AMENDMENT AND RESPONSE TO OFFICE ACTION****Remarks****Restriction Requirement and Double Patenting Requirements**

The examiner has made inconsistent restriction and double patenting rejections. In the parent application, now U.S. Patent No. 6,342,218, the claims were not restricted between method of treatment and composition. In this case, the examiner has declared the method and composition two separate inventions (which by definition cannot be restricted over the other), then rejected claims 8-10, and 12-14 as obvious over claims 7-12 of the earlier issued patent.

Solely to facilitate prosecution, enclosed with this amendment is a Terminal Disclaimer to U.S. Patent No. 6,342,218. However, it is respectfully requested that the examiner reconsider the restriction requirement. If the composition claims in the first patent make obvious the composition claims in this application, and the method of use was not a separate invention in the first case, how can the method of use in this case be a totally distinct invention? Consideration of all claims on the merits is earnestly solicited.

**Rejection Under 35 U.S.C. § 112, first paragraph**

Claims 8-10 and 12-14 were rejected under 35 U.S.C. § 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the art that the inventor had possession of the claimed invention. Applicants respectfully traverse this rejection to the extent that it is applied to the claims as amended.

U.S.S.N. 09/768,155

Filed: January 23, 2001

**AMENDMENT AND RESPONSE TO OFFICE ACTION**

While it is believed that the specification provides support for the phrase "prevent the anti-dsDNA antibodies from interfering with protein synthesis", claim 8 has been amended to delete the phrase. Example 2 clearly suggests that the function of anti-dsDNA antibodies is to interfere with protein synthesis. Blocking antibodies have been known in the art since the 1970's and it was very well known that making an anti-idiotypic antibody could block the function of the primary antibody. Furthermore, one of skill in the art would have read Examples 2 and 3 in view of the teachings of the specification on pages 9-12 to know that the anti-idiotypic antibodies would prevent function of the anti-dsDNA antibodies in disrupting protein synthesis.

Claim 8 now recites:

A therapeutic composition in a pharmaceutically acceptable carrier for administration to a human patient having anti-dsDNA antibodies (page 9, lines 22-23); comprising

single chain anti-idiotypic antibody fragments (page 9, lines 25-34; page 10, lines 11-19 and 23-page 12, line 11);

immunoreactive with anti-dsDNA antibodies isolated from SLE patients (page 5, lines 22-26).

Explicit support for each element is indicated in parenthesis.

Support for new claim 15 is found at page 10, lines 23-28. This is the embodiment that is to be in clinical trials later this year.

U.S.S.N. 09/768,155

Filed: January 23, 2001

**AMENDMENT AND RESPONSE TO OFFICE ACTION****Rejections Under 35 U.S.C. § 102**

Claims 8-10 and 12-14 were rejected under 35 U.S.C. § 102(e) as being anticipated by U.S. Patent No. 6,232,444 to Weisbart ("Weisbart"). Applicants respectfully traverse this rejection to the extent that it is applied to the claims as amended.

Weisbart discloses a murine monoclonal antibody, and states that one could make equivalent antibodies of any species of origin. This is not single chain nor is there any disclosure of how to make a single chain antibody - or that the binding of such an antibody fragment would effectively bind to anti-dsDNA antibodies. Neither can it be administered to a human as a human therapeutic. Therefore Weisbart does not anticipate the claimed subject matter. The only reference to "human" is with respect to the anti-dsDNA antibodies which are bound by the murine monoclonal antibodies (col. 3, lines 17-23, first describes the murine monoclonal - then says that it is specific an an idiotypic or anti-DNA antibodies - which can be of any species - allowing the murine monoclonal to be used as a diagnostic). There is no method for making human antibodies since the only method which is disclosed (col. 2, lines 29-58) to make antibodies requires immunization with the anti-dsDNA antibodies. One could not legally immunize a human with such an antigen, nor is there any indication that such an immunization would be effective, since lupus patients are characterized by anti-dsDNA antibodies and there is no evidence they produce antibodies to these antibodies.

Enclosed is a copy of applicants' publication describing the production of the single chain recombinant human antibodies. It required cloning the human genes from cells isolated

U.S.S.N. 09/768,155

Filed: January 23, 2001

**AMENDMENT AND RESPONSE TO OFFICE ACTION**


from lupus patients, then making them recombinantly, Zhang, et al. *Lupus* 11, 362-369(2002).

Applicants isolated three different clones. Unlike the antibodies of Weisbart which bind at most 59% of lupus anti-dsDNA antibodies (see col. 5, lines 43-53), these antibodies, made from any of the three different clones that were isolated from humans, bind 100% of the patients' anti-dsDNA antibodies. It is this that makes these antibodies useful as therapeutics.

Therefore Weisbart does not anticipate, nor make obvious, the claimed subject matter.

Allowance of all pending claims is earnestly solicited.

Respectfully submitted,

  
Patrea L. Pabst  
Reg. No. 31,284

Date: May 29, 2003

HOLLAND & KNIGHT LLP  
One Atlantic Center, Suite 2000  
1201 West Peachtree Street  
Atlanta, Georgia 30309-3400  
(404) 817-8473  
(404) 817-8588 (Fax)

Lupus (2002) 11, 362-369  
www.lupus-journal.com

## PAPER

# Production and characterization of human monoclonal anti-idiotypic antibodies to anti-dsDNA antibodies

W Zhang<sup>1</sup>, MB Frank<sup>1</sup> and M Reichlin<sup>1\*</sup>

<sup>1</sup>Oklahoma Medical Research Foundation, Oklahoma City, Oklahoma, USA

Anti-dsDNA autoantibodies are the hallmark of systemic lupus erythematosus (SLE) and frequently correlate with disease activity. In this study we report the isolation and characterization of human anti-Id monoclonal antibody fragments as single-chain Fv fragments (scFv) against anti-dsDNA antibody. The anti-Id monoclonal antibodies, specific for anti-dsDNA antibodies, have been cloned from phage display antibody scFv libraries derived from a patient with SLE. The V gene repertoires were derived from the RNA obtained from the B cells of an SLE patient with anti-Ro/SSA and anti-La/SSB antibodies. Affinity-purified anti-dsDNA antibodies were used for selection of bacterial clones producing specific scFv antibody fragments against anti-dsDNA antibodies and little reactivity with normal IgG and other IgG antibodies by ELISA. The anti-Id antibody recognizes a public idiotype that is broadly cross-reactive with polyclonal and monoclonal anti-dsDNA antibodies. This binding was largely inhibited by dsDNA antigen. The anti-Id antibody inhibited anti-dsDNA binding to dsDNA antigen in immunoassays and in the *Crithidia lucillae* assay. The anti-Id scFv antibody fragments derived from human genes could modulate the pathogenicity of anti-dsDNA autoantibodies and may have therapeutic implications in SLE. They may also be used as probes in studies of the structure of the idiotype. *Lupus* (2002) 11, 362-369.

**Key words:** monoclonal antiidiotype to anti-dsDNA

## Introduction

Systemic lupus erythematosus (SLE) is a clinically diverse autoimmune disease with potential for serious organ dysfunction. SLE is characterized by polyclonal B cell activation of antibodies directed against a broad range of self antigens such as DNA. Antibodies to double-stranded DNA (anti-dsDNA) are believed to participate in tissue damage, which cause clinical features of disease,<sup>1</sup> particularly glomerulonephritis in SLE. Anti-dsDNA antibodies are deposited in the kidneys of lupus patients<sup>2,3</sup> and levels of IgG anti-dsDNA antibodies correlate with the degree of renal damage detectable histologically.<sup>4</sup> The capacity of anti-dsDNA antibodies to form immune deposits may be influenced by their idiotypes.<sup>5</sup> The role of the Id network has been studied extensively in SLE.

The specific suppression of undesirable immune responses may be accomplished by the regulation of idiotype (Id)—anti-Id interactions, as postulated by Jerne.<sup>6</sup> Ids are defined as unique arrangements of idiotopes (epitopes) present on heavy and light chains of immunoglobulins. Ids may be either 'private' or 'public'. Private Ids are unique idiotope regions on immunoglobulin molecules from a single B cell clone, whereas public Ids are common idiotope regions on immunoglobulins of either the same Id in different individuals or even antibodies of different specificities. In the on-going interactions between the immune system and antigens, antibodies are generated with either private or public Ids. As the immune system recognizes these idiotopes, anti-Id antibodies are produced. In previous experiments, we developed methods that isolated anti-anti-dsDNA (anti-Id) antibody (Ab) from sera with anti-dsDNA and from sera with anti-Ro/SSA and anti-La/SSB precipitins in SLE. All anti-Ids have the potential as immunologically specific reagents to block and/or down-regulate anti-dsDNA antibodies in SLE.<sup>7</sup> The aim of this study was to produce and characterize monoclonal anti-Id

\*Correspondence: M Reichlin, Oklahoma Medical Research Foundation, Oklahoma City, OK 73104, USA.

E-mail: Morris-Reichlin@omrf.ouhsc.edu

Received 10 December 2001; accepted 20 March 2002

© Arnold 2002

antibodies to anti-dsDNA antibodies by the phage display method.

Molecular approaches for the generation of monoclonal (mAbs) offer several advantages over traditional methods such as EBV (Epstein-Barr virus) transformation of B cells or hybridoma technology. Molecular genetic approaches allow the use of genetic material from any source of available B lymphocytes to create random combinations of cloned heavy and light chain Ig genes. The display and selection of antibody fragments on the surface of phage mimics immune selection, and antibodies have also been isolated without immunization, from repertoires of V genes rearranged *in vivo* or *in vitro*.<sup>8,9</sup> The same phage repertoire may be used to generate many different binding specificities, including those that are difficult to raise by immunization, for example against self-antigens. In this study, we have isolated anti-idiotypic antibody from a phage display library which reacts specifically with anti-dsDNA antibodies from SLE patients.

## Materials and methods

### *Preparation of IgG anti-dsDNA antibody and preparation of IgG F(ab)<sub>2</sub> fragments*

The IgG fraction from one anti-dsDNA positive serum (AP) was isolated by chromatography on DE-52 and protein A-sepharose (Sigma). Purified IgG from either DE-52 or protein A was passed through a dsDNA affinity column (Sigma). The column was washed with Tris buffered saline (pH 7.4), and bound protein was eluted with 3 M MgCl<sub>2</sub>. F(ab)<sub>2</sub> fragments were prepared from purified IgG anti-dsDNA antibodies by pepsin digestion.<sup>10</sup> Human polyclonal antiidiotypic antibody was prepared as described previously.<sup>7</sup>

### *Patient data*

A lupus patient with anti-Ro/SSA and anti-La/SSB precipitins was the donor of the cells used in the preparation of cDNA.

### *Construction of the IgG-derived V gene phage display library*

Blood from patient JN was used to separate white blood cells (PBLs) over Ficoll. The RNA was prepared using a High Purity RNA Isolation Kit (Boehringer, Mannheim, Germany). The cDNA synthesis was carried out for V<sub>H</sub>, V<sub>LK</sub> and V<sub>LA</sub> separately by diluting 4 µg of total RNA into a 45 µl reaction mixture, resulting in a 50 µl reaction mixture containing 140 mM KCl, 50 mM Tris-HCl pH 8.1, 8 mM

MgCl<sub>2</sub>, 10 mM DTT, 250 mM of each dNTP (dATP, dGTP, dTTP and dCTP), 10 pmol relevant constant region primers for IgG, C<sub>K</sub>, and C<sub>L</sub>, respectively, and first-strand cDNA was synthesized. The mixture was heated to 67°C for 5 min before 80 U of human placental RNase inhibitor and 50 U of avian myeloblastosis virus (AMV) reverse transcriptase were added. The mixture was incubated at 42°C for 1 h, heated at 100°C for 3 min, quenched on ice and centrifuged for 5 min.

Polymerase chain reaction (PCR) was used to amplify V<sub>H</sub>, V<sub>LK</sub> and V<sub>LA</sub> genes. For primer sequences, see Marks *et al.*<sup>11</sup> Reaction mixtures (50 µl) were prepared containing 5 µl cDNA, 20 pmol of each forward and reverse primer (equimolar mixture of the family-specific primer), 20 µl dNTP-Mix, 10 mM KCl, 10 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 20 mM Tris-HCl pH 8.8, 20 mM MgCl<sub>2</sub>, 100 µg bovine serum albumin (BSA)/ml and 1 U cetus DNA polymerase. The reaction mixture was cycled 30 times (94°C 1 min, 60°C 1 min and 72°C 1.5 min) with flanking primers containing different restriction sites. The cloning into PHENIX was carried out in two steps. The V<sub>H</sub> genes were ligated into PHENIX cut with NcoI and SalI and electroporated into *E. coli* strain TG1. Then, DNA containing the V<sub>L</sub> library was prepared and each of the fragments containing either V<sub>K</sub> or V<sub>L</sub> genes were cloned into PHENIX-V<sub>H</sub> cut with Apa I and Not I and electroporated separately into *E. coli* TG1.

### *Soluble expression of scFv*

Single ampicillin-resistant colonies were picked for the production of soluble scFv fragments according to Marks *et al.*<sup>11</sup>

### *Selection*

The phage repertoire was panned using Nunc immunotubes. Anti-dsDNA IgG Fab<sub>2</sub> was coated overnight at 4°C at a concentration of 20 µg/ml in PBS.<sup>11,12</sup>

### *ELISA*

Single ampicillin-resistant colonies were screened to identify those producing antigen-binding scFv by ELISA. The IMMULON plate was divided into two. One half was coated with affinity purified anti-dsDNA Fab<sub>2</sub> Ab at 10 µg/ml, while the other half of the plate was coated with the same concentration of control normal IgG F(ab)<sub>2</sub>. Wells were washed three times with PBS and blocked with 100 µl of 3% BSA in PBS for 2 h at room temperature. Wells were washed three times and 50 µl/ml bacterial supernatant containing expressed scFv protein was added to both sides of the

plate. Incubation was for 1 h at RT. Wells were washed three times and 50  $\mu$ l/well of anti-VSV-G (clone P5D4) tag mouse monoclonal antibody was added at 1  $\mu$ g/ml in PBS. Incubation was at RT for 1 h. Wells were washed three times and conjugated anti-mouse monoclonal antibody at 1  $\mu$ g/ml in PBS was added. Incubation was at room temperature (RT) for 1 h. Wells were washed three times, substrate was added and plates were read at 410 nm.

#### *Purification of scFv*

The scFv fragments were purified by Protein LA agarose column and characterized by SDS-PAGE and Western blot.

#### *Control scFv antibody*

Anti-U1 A (SPA1) clone was the generous gift of Dr WJ Van Venrooij. This phage display scFv antibody is directed toward U1 A protein.

#### *ELISA for detecting the Id on purified human IgG anti-dsDNA antibodies*

IMMULON plates were used to detect the Id on five affinity purified human IgG anti-dsDNA antibodies isolated from SLE patients sera, three human IgG anti-dsDNA monoclonal antibodies, and normal IgG as control. The ELISA plate was coated with purified scFv (5  $\mu$ g/ml) and purified anti-dsDNA Ab was applied to the plate. Binding of IgG was detected using goat anti-human IgG Fc fragment specific alkaline phosphatase conjugate (Sigma).

#### *Inhibition*

*Inhibition of anti-dsDNA binding to anti-Id Ab by dsDNA antigen.* The method has been described.<sup>13</sup> Reaction mixtures of anti-dsDNA antibody at the same concentration and antigen at varying concentrations were prepared. The ELISA plate was coated by purified scFv (5  $\mu$ g/ml). Calf thymus dsDNA and yeast transfer RNA were incubated for 2 h with anti-dsDNA antibodies at RT before being transferred to anti-Id coated wells. The amount of human antibodies bound to the plate was determined using an anti-human IgG Fc specific alkaline phosphatase conjugate (Sigma).

*Inhibition of anti-dsDNA antibodies binding to dsDNA by anti-Id Ab.* ELISA plate was coated with dsDNA antigen (20  $\mu$ g/ml). Reaction mixtures of anti-dsDNA Ab at the same concentration and anti-Id Ab at varying concentrations were prepared. The mixtures were incubated for 2 h at RT before being transferred to dsDNA coated wells. The amount of

anti-dsDNA Ab bound to the plate was determined by using an anti-human IgG Fc specific alkaline phosphate conjugate (Sigma).

*Immunofluorescent staining and analysis.* An indirect fluorescent antibody test utilizing *Critidia luciliae* to detect and titer the presence of anti-dsDNA antibody test kit (HELIX diagnostic) was used. Fluid-phase inhibition of anti-dsDNA binding by anti-Id was performed at RT for 2 h prior to staining. Anti-Id samples and controls were added to slide wells and incubated for 30 min at RT, followed by PBS washes. Fluorescent goat anti-human IgG was added and incubated for 30 min and then washed. After adding the coverslip, the slides were read with a fluorescent microscope.

## Results

#### *Selection of human anti-anti-dsDNA antibody fragment*

The phage display library containing the cDNA from the PBLs of patient (JN) with anti-Ro/SSA and anti-La/SSB antibody was screened for the presence of human antibody fragments directed to the IgG anti-dsDNA antibody (AP) and normal IgG as the control. The clones producing anti-anti-dsDNA reactive scFv fragments were observed after a single round of selection, and the number of positive clones increased with each subsequent round. In all, 96 individual bacterial clones were induced to produce soluble scFv fragments which were then tested for binding to the selecting antigen in the ELISA. PCR fingerprint analysis of the V gene cassette showed a unique BstNI digestion pattern. The V regions of selected scFv fragments were determined from double-stranded DNA purified with QIAGEN kit, and sequencing was done in an automatic sequencer. Anti-anti-dsDNA antibody was isolated from patient-derived libraries by four rounds of selection, using purified anti-dsDNA antibody (AP) as antigen. The specificity of the antibodies was determined by ELISA. Two antibody clones (B12, A1) reacted with anti-dsDNA antibodies and neither clone reacted with Ro/SSA and La/SSB antigens in ELISA. All the work in this paper was performed with the B12 scFv.

#### *Protein purification and analysis of binding affinity of soluble scFv anti-Id antibody*

The scFv antibody was purified in one step on a protein LA agarose column (Clontech). The purified scFv fragment was detected by Western blot (Figure

1), using the anti-tag (anti-VSV) monoclonal antibody to identify the scFv 30 kDa protein.

The purified anti-dsDNA antibodies (AP) were coated on an ELISA plate for testing purified scFv binding, while a human anti-Id polyclonal Ab isolated from a SLE patient's serum served as a positive control. Normal IgG and the clone producing anti-U1 A scFv antibody served as un-reactive controls. As seen in Table 1 and in Figure 2, purified anti-Id binds to anti-dsDNA antibody more strongly than to normal IgG while anti-U1 A scFv antibody binds similarly to normal IgG and anti-dsDNA antibody.

We also used anti-Id scFv antibody to coat the ELISA plate for testing different anti-dsDNA antibody preparations binding to anti-Id Ab. As seen in Figure 3, five different affinity-purified polyclonal human anti-dsDNA populations were shown to bind the anti-Id (B12) scFv Ab more strongly than did normal IgG. Three human monoclonal antibodies also bound anti-Id (B12) scFv fragment more strongly than did normal IgG. Affinity purified IgG anti-Ro/SSA,

anti-La/SSB, anti-U1RNP and normal IgG bound anti-Id poorly and equally. The Id detected appears to be clearly present on the IgG anti-dsDNA but not on the IgG anti-Ro/SSA, anti-La/SSB, anti-U1RNP or normal IgG. To show the generality of these findings an additional 17 samples of anti-dsDNA were assayed in their ability to react with the anti-Id B12, as seen in Figure 4.

#### Characterization of the Id on IgG anti-dsDNA Ab

The IgG anti-dsDNA antibodies were studied for the ability of cognate antigen to inhibit binding to the anti-Id reagent. The rationale for this assay was that dsDNA should be able to block the reaction if the idiotype was an intrinsic part of the antibody binding site. The results of the inhibition experiments are

Table 1 Reactivity of purified anti-dsDNA antibodies with anti-Id reagents, isolated human anti-Id antibodies and scFv anti-U1 A protein antibody in ELISA

| Purified antibodies                       | Coated with anti-dsDNA F(ab) <sub>2</sub> (10 µg/ml) | Coated with CFII F(ab) <sub>2</sub> (10 µg/ml) |
|---|--|--|
| B12 (scFv) anti-Id antibody               | 1.19   | 0.20   |
| AI (scFv) anti-Id antibody                | 1.02   | 0.15   |
| IgG anti-Id antibodies from patient serum | 1.15   | 0.30   |
| Anti-U1 A protein (scFv)                  | 0.19   | 0.13   |

#### Western blot of scFv Ab

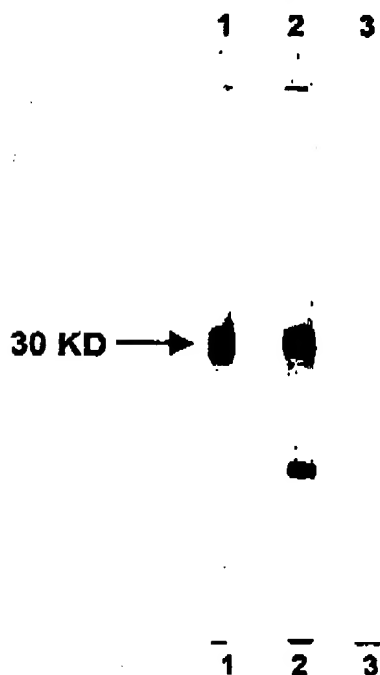


Figure 1 Western blot of scFv Ab. Western blotting of immunoreactive protein from soluble scFv Ab. Lane 1—purified anti-U1A protein scFv antibody was electrophoresed on a 15% SDS-PAGE gel. Lane 2—purified anti-Id (B12) scFv antibody was electrophoresed on a 15% SDS-PAGE gel. Lane 3—bacterial supernatant not containing scFv fragment as control. Lanes 1, 2 and 3 were probed with mouse anti-VSV tag monoclonal antibody.

#### scFv(B12) binding to anti-dsDNA antibody

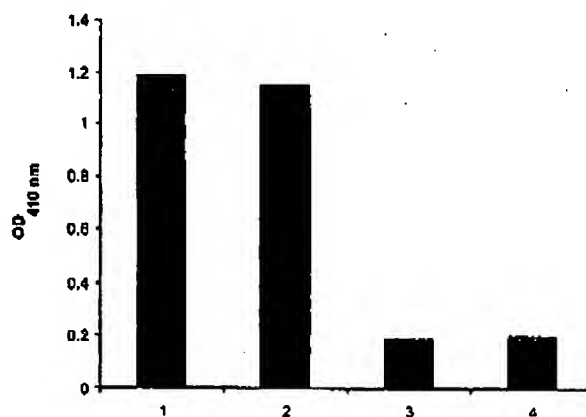


Figure 2 scFv (B12) binding to anti-dsDNA antibody. ELISA plate was coated with anti-dsDNA Fab<sub>2</sub> (10 µg/ml). Results expressed as mean optical density (OD)  $\pm$  s.d. 1, Anti-Id scFv antibody (B12) ( $1.19 \pm 0.021$ ); 2, human anti-Id antibody from patient serum ( $1.15 \pm 0.212$ ); 3, CFII IgG ( $0.19 \pm 0.141$ ); 4, anti-U1 A scFv antibody ( $0.2 \pm 0.070$ ). Goat anti-human Fc conjugate was used to detect binding of human anti-Id antibody and CFII IgG, and mouse anti-VSV conjugate was used to detect scFv binding.



## Anti-dsDNA and control antibodies reacting with B12

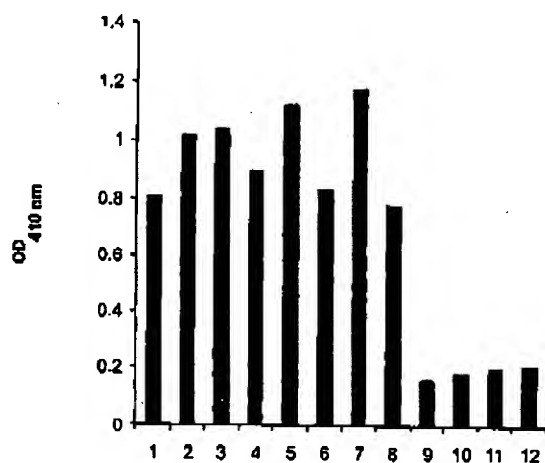


Figure 3 Anti-dsDNA and control antibodies reacting with B12. Anti-dsDNA and control antibodies were assayed by Id-specific ELISA. The ELISA plate was coated with 5 µg/ml anti-Id (B12) antibody. Different affinity purified antibodies were applied to the plate at an IgG concentration of 5 µg/ml. Results expressed as mean OD ± s.d. 1, A.P. polyclonal anti-dsDNA antibody (0.81 ± 0.042); 2, S.D. polyclonal anti-dsDNA antibody (1.02 ± 0.183); 3, S.L. polyclonal anti-dsDNA antibody (1.04 ± 0.084); 4, P.C. polyclonal anti-dsDNA antibody (0.9 ± 0.042); 5, D.S. polyclonal anti-dsDNA antibody (1.12 ± 0.099); 6, RH14 monoclonal anti-dsDNA antibody (0.84 ± 0.042); 7, 32.B9 monoclonal anti-dsDNA antibody (1.18 ± 0.198); 8, 35.21 monoclonal anti-dsDNA antibody (0.78 ± 0.042); 9, CFII IgG (0.16 ± 0.070); 10, D.S. polyclonal anti-Ro/SSA antibody (0.18 ± 0.042); 11, K.A. polyclonal anti-La/SSB antibody (0.2 ± 0.056); 12, 1356 polyclonal anti-U1 RNP (0.21 ± 0.084).

plotted in Figure 5. As seen, 5 µg/100 µl DNA can inhibit the binding of three anti-dsDNA antibody preparations from 55 to 80%. As a control we used yeast transfer RNA, which was not effective in inhibiting this interaction. The scFv anti-Id antibody was also studied for blocking anti-dsDNA antibody activity. As seen in Figure 6, the concentration of scFv fragment required to inhibit the anti-dsDNA antibody to dsDNA antigen by 50% of maximal binding was about five times higher than the dsDNA antibody concentration.

An indirect fluorescent antibody test utilizing *Crithidia luciliae* was used as an additional method to detect and titer the presence of anti-dsDNA antibody. We have shown that the binding of anti-dsDNA antibody binding to the crithidia kinetoplast can be effectively inhibited by anti-Id scFv antibody, as seen in Figure 7. Indeed as seen, 1 µg/50 µl of anti-Id scFv antibody partially inhibited binding and 4 µg/50 µl of anti-Id scFv antibody completely inhibited binding.

These data taken together attest to the specific reactivity of the scFv fragments with the binding site of anti-dsDNA antibody.

## Anti-dsDNA and control antibodies reacting with anti-Id Ab

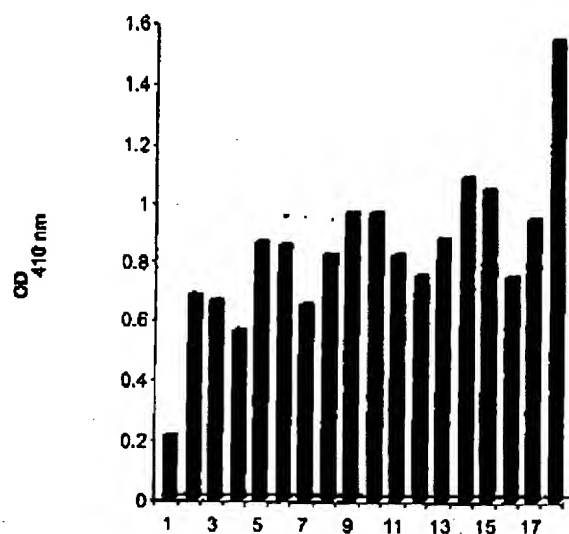
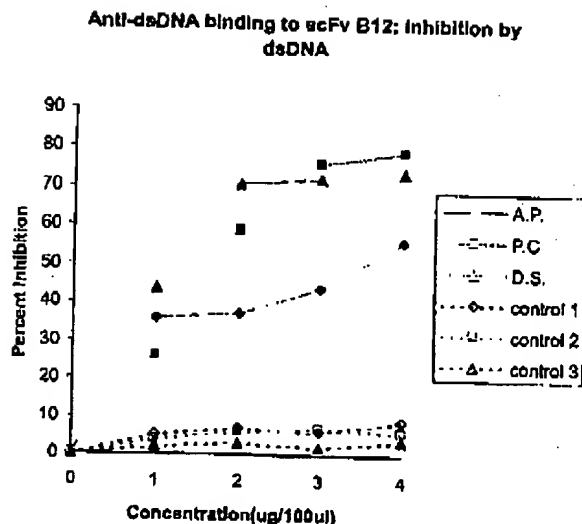


Figure 4 The additional 17 anti-dsDNA and control antibodies were assayed by Id-specific ELISA. The ELISA plate was coated with 5 µg/ml anti-Id (B12) antibody. Different affinity-purified antibodies were applied to the plate at an IgG concentration of 5 µg/ml. Results expressed as mean OD ± s.d. 1, CFII (0.21 ± 0.070); 2, F.E. polyclonal anti-dsDNA antibody (0.68 ± 0.056); 3, F.C. polyclonal anti-dsDNA antibody (0.66 ± 0.056); 4, R.R. polyclonal anti-dsDNA antibody (0.56 ± 0.014); 5, S.R. polyclonal anti-dsDNA antibody (0.86 ± 0.056); 6, A.I. polyclonal anti-dsDNA antibody (0.85 ± 0.028); 7, H.J. polyclonal anti-dsDNA antibody (0.65 ± 0.070); 8, T.J.A. polyclonal anti-dsDNA antibody (0.82 ± 0.028); 9, W.C. polyclonal anti-dsDNA antibody (0.97 ± 0.113); 10, B.J. polyclonal anti-dsDNA antibody (0.97 ± 0.04); 11, D.B. polyclonal anti-dsDNA antibody (0.82 ± 0.098); 12, N.S. polyclonal anti-dsDNA antibody (0.73 ± 0.070); 13, J.F. polyclonal anti-dsDNA antibody (0.88 ± 0.098); 14, H.E. polyclonal anti-dsDNA antibody (1.09 ± 0.282); 15, R.P. polyclonal anti-dsDNA antibody (1.05 ± 0.056); 16, J.P. polyclonal anti-dsDNA antibody (0.75 ± 0.084); 17, E.H. polyclonal anti-dsDNA antibody (0.95 ± 0.010); 18, C.W. polyclonal anti-dsDNA antibody (1.55 ± 0.120).

## Discussion

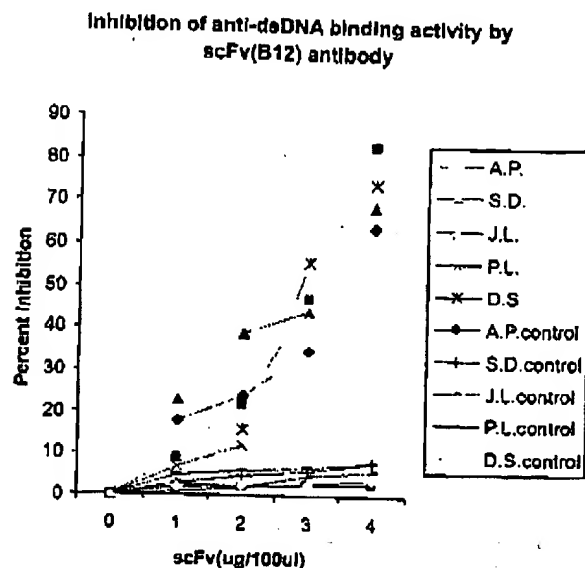
We previously reported on methods of isolation of anti-Id antibodies to anti-dsDNA antibodies from sera with anti-Ro/SSA/La/SSB precipitins as well as from sera with anti-Ro/SSA and anti-U1 RNP precipitins in SLE.<sup>7,14</sup> All anti-Id antibodies have the potential to immunologically specific reagents to block and/or down-regulate anti-dsDNA antibodies in SLE patients.

We report here the isolation and characterization of human anti-Id monoclonal antibody fragments (scFv) against anti-dsDNA antibodies. The antibodies specific for anti-dsDNA antibodies have been cloned from a phage-display antibody scFv library derived from a patient with SLE. The anti-Id antibody showed broad but specific cross-reactivity with several different polyclonal and monoclonal anti-dsDNA antibodies



**Figure 5** Anti-dsDNA binding to scFv B12: inhibition by dsDNA. ELISA plate was coated with 5  $\mu$ g/ml purified anti-Id (B12). Anti-dsDNA antibody mixed with dsDNA at various concentrations using yeast RNA as control. 1, A.P. polyclonal anti-dsDNA antibody; 2, P.C. polyclonal anti-dsDNA antibody; 3, D.S. polyclonal anti-dsDNA antibody.

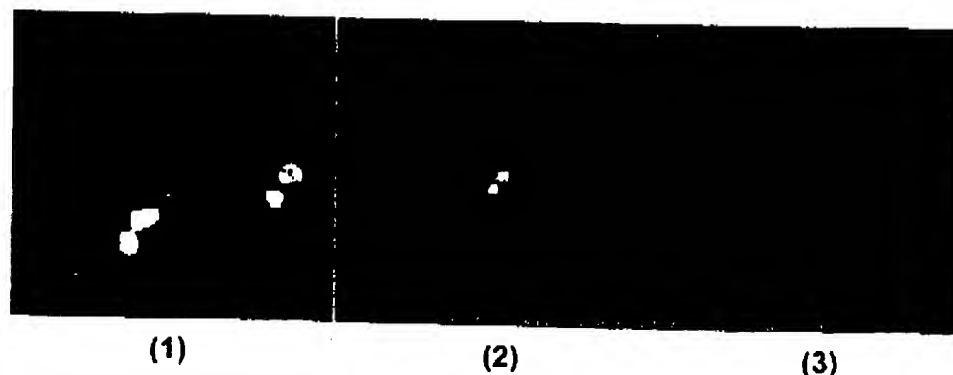
from unrelated individuals. We established a patient-derived V gene phage display library using a PHENIX-derived phagemid vector permitting the independent cloning of the  $V_H$  and  $V_L$  gene repertoires. The V gene repertoires were derived from the RNA obtained from the PBLs of an SLE patient with anti-Ro/SSA and anti-La/SSB antibodies. Interestingly, neither of the isolated clones had anti-Ro/SSA or anti-La/SSB activity. The cloning strategy was designed to maintain the diversity of the repertoire. The sequence diversity was assessed,



**Figure 6** Inhibition of anti-dsDNA binding activity by scFv (B12) antibody. ELISA plate was coated with 10  $\mu$ g/ml dsDNA. Anti-dsDNA antibody mixed with B12 at various concentrations using anti-UI scFv as control. 1, A.P. polyclonal anti-dsDNA antibody; 2, S.D. polyclonal anti-dsDNA antibody; 3, J.L. polyclonal anti-dsDNA antibody; 4, P.L. polyclonal anti-dsDNA antibody; 5, D.S. polyclonal anti-dsDNA antibody.

and it was demonstrated that all major  $V_H$  families were represented combined with a diverse set of  $V_L$  genes. After using purified anti-dsDNA antibodies, selection of bacterial clones was accomplished producing a specific scFv antibody fragment against anti-dsDNA antibodies and no reactivity with normal and other IgG antibodies as seen by ELISA. Its binding to anti-dsDNA Ab was largely inhibitable

#### Inhibition of anti-dsDNA binding by anti-Id (B12) binding by anti-Id (B12)



**Figure 7** Inhibition of anti-dsDNA antibody binding to cells by scFv anti-Id (B12). 1, Anti-dsDNA antibody (0.5  $\mu$ g/50  $\mu$ l) stains cells; 2, anti-dsDNA antibody was inhibited by B12 (1  $\mu$ g/50  $\mu$ l); 3, anti-dsDNA antibody was inhibited by B12 (4  $\mu$ g/50  $\mu$ l).

by dsDNA antigen. This anti-Id was functional; not only did it inhibit anti-dsDNA activity in immunochemical assays, but also inhibited anti-dsDNA binding to cells. These characteristics are analogous to those observed with polyclonal human anti-Ids. The origin of autoantibodies in SLE is unknown, and the mechanisms that account for the diversity of serologic abnormalities in the disease are poorly understood. IgG autoantibodies to dsDNA appear to play a prominent role in lupus nephritis. Several groups have shown binding of anti-dsDNA antibodies to live cells,<sup>13,15-17</sup> and in some instances penetration with nuclear localization. Anti-dsDNA antibodies appear to be very polyreactive and react with a variety of intra-cellular proteins such as Sm/RNP, vimentin, laminin and heparan sulfate. Such information may prove invaluable for the design of antibodies as drug delivery systems for the transport of an intracellular therapeutic agent. In patients who spontaneously recover from autoimmune diseases, anti-idiotypes may play an important role. In fact, autologous anti-idiotypes against autoantibodies have been demonstrated in the sera of patients experiencing remission in a variety of autoimmune disorders.<sup>18-25</sup>

We have produced a novel phage display anti-Id reagent that will enhance our understanding of the regulation and pathogenicity of anti-dsDNA antibodies in SLE. Anti-dsDNA antibodies bearing a major cross-reactive Id in SLE patients have their binding to dsDNA inhibited by anti-Id scFv antibody fragments *in vitro*.

### Acknowledgements

The authors are grateful to Drs Joachim Kalden and Thomas Winkler of Erlangen University in Germany for the gift of the monoclonal anti-dsDNA antibody preparations 32 B9 and 35.21, and to Dr David Isenberg for his gift of the human monoclonal anti-dsDNA antibody RH 14. The authors also appreciate the gift of the scFv anti-U1 A protein by Dr WJ van Venrooij of Nijmegen University in The Netherlands.

### References

- Isenberg OA, Ravirajan CT, Rasmussen A, Kalsi J. The role of antibodies to DNA in systemic lupus erythematosus. *Lupus* 1997; 6: 290-304.
- Winfield JB, Faerman I, Koffler D. Avidity of anti-DNA antibodies in serum and IgG glomerular elutes from patients with system lupus erythematosus. Association of high avidity anti-native DNA antibody with glomerulonephritis. *J Clin Invest* 1977; 59: 90-96.
- Koffler D, Schur PH, Kunkel HG. Immunological studies concerning the nephritis of systemic lupus erythematosus. *J Exp Med* 1967; 126: 607-624.
- Okamura M, Kanayama Y, Anasui K et al. Significance of enzyme linked immunosorbent assay (ELISA) for antibodies to double stranded and single stranded DNA in patients with lupus nephritis; correlation with severity of renal histology. *Ann Rheum Dis* 1993; 52: 14-20.
- Vlahakos DV, Foster MH, Adams S et al. Anti-DNA antibodies form immune deposits at distinct glomerular and vascular sites. *Kidney Int* 1992; 41: 1690-1700.
- Jerne NK. Towards a network theory of the immune system. *Ann Immunol (Paris)* 1974; 125C(1-2): 373-389.
- Zhang W, Winkler T, Kalden J R, Reichlin M. Isolation of human anti-idiotypes broadly cross-reactive with anti-dsDNA antibodies from patients with systemic lupus erythematosus. *Scand J Immunol* 2001; 53: 192-197.
- Larick JW, Danielsson L, Brenner CA, Abrahamson M, Fry KE, Boorebeck CA. Rapid cloning of rearranged immunoglobulin genes from human hybridoma cells using mixed primers and the polymerase chain reaction. *Biochem Biophys Res Commun* 1989; 160: 1250-1256.
- Mullinax RL, Gross EA, Amberg JR et al. Identification of human antibody fragment clones specific for tetanus toxoid in a bacteriophage lambda immunocopy library. *Proc Natl Acad Sci USA* 1990; 87: 8095-8099.
- Nisonoff A, Wissler FC, Lipman LN, Woenley DL. Separation of univalent fragments from the bivalent rabbit antibody molecule by reduction of disulfide bonds. *Arch Biochem Biophys* 1960; 89: 230-244.
- Marks JD, Hoogenboom HR, Bonnett TP, McCafferty JM, Griffiths AD, Winter G. By-passing immunization human antibodies from V-gene libraries displayed on phage. *J Mol Biol* 1991; 222: 581-597.
- Pedrollo FE, Fisch I, Wieslander J, Marks JD, Lockwood CD, Ourwand WH. Human autoimmune anti-proteinase 3 scFv from a phage display library. *Clin Exp Immunol* 1997; 107: 267-281.
- Rekvig OP, Hannestad K. The specificity of autoantibodies that react with both cell nuclei and plasma membrane: the nuclear antigen is present on core mononucleosomes. *J Immunol* 1979; 123: 2673-2688.
- Zhang W, Reichlin M. Some autoantibodies to Ro/SS-A and La/SS-B are anti-idiotypes to anti-double-stranded DNA. *Arthritis Rheum* 1996; 39: 522-531.
- Alarcon-Segovia D, Llorente L. Antibody penetration into living cells. IV. Different effects of anti-native DNA and anti-ribonucleoprotein IgG on the cell cycle of activated T cells. *Clin Exp Immunol* 1983; 52: 365-371.
- Okudaira K, Yoshizawa H, Williams RC Jr. Monoclonal murine anti-DNA antibody interacts with living mononuclear cells. *Arthritis Rheum* 1987; 30: 669-678.
- Koren E, Koscew M, Wolfson-Reichlin M et al. Murine and human antibodies to native DNA that cross react with the A and D SnRNP polypeptides cause direct injury of cultured kidney cells. *J Immunol* 1995; 154: 4857-4864.
- Watts RA, Ravirajan CT, Staines NA. A human fetal monoclonal DNA-binding antibody shares idiotypes with fetal and adult murine monoclonal DNA-binding antibodies. *Immunology* 1990; 69: 348-354.
- Abdou NI, Wall H, Lindsley HB, Halsey JF, Suzuki T. Network theory in autoimmunity: in vitro suppression of serum anti-DNA antibody to DNA by anti-idiotypic antibody in systemic lupus erythematosus. *J Clin Invest* 1980; 67: 1297-1304.
- Lefvert AK. Anti-idiotypic antibodies against the receptor antibodies in myasthenia gravis. *Scand J Immunol* 1981; 12: 493-497.
- Dwyer DS, Bradley RJ, Urquhart CK, Kearney JF. Naturally occurring anti-idiotypic antibodies in myasthenia gravis patients. *Nature* 1983; 301: 611-614.
- Pasquali JL, Urlacher A, Storek D. Idiotype network: possible explanation of seronegativity in a patient with rheumatoid arthritis. *Clin Exp Immunol* 1984; 55: 281-286.
- Sikorska HM. Anti-thyroglobulin anti-idiotypic antibodies in sera of patients with Hashimoto's thyroiditis and Graves disease. *J Immunol* 1986; 137: 3786-3795.

*Monoclonal anti-idiotypes to anti-dsDNA antibodies*  
W Zheng et al

- 24 Sultan Y, Rossi F, Kazatchkine MD. Recovery from anti-VIII:C (anti-hemophilic factor) autoimmune disease is dependent on generation of anti-idiotypes against anti-idiotypes against anti-VIII:C autoantibodies. *Proc Natl Acad Sci USA* 1987; 84: 838-831.

- 25 Muryoi T, Sasaki T, Harata N, Takai O, Tamata E, Yoshinaga K. Heterogeneity of anti-idiotypic antibodies to anti-DNA antibodies in humans. *Clin Exp Immunol* 1988; 71: 67-72.

369